Taxonomy of endosymbiotic bacteria from a novel *Lupinus sp.* (*Lupinus mariae-josephi*) endemic of a limed-alkaline soil habitat in Southeastern Spain.

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Lupinus mariae-josephi is a recently described *Lupinus* species (Pascual 2004) endemic of a Southeastern area of Spain with soils singularly of high pH and active lime content where it is endangered due to the reduced size of its habitat. Ten isolates of *L. mariae-josephi* endosymbiotic bacteria were obtained using trap-plants and soils from five sampling points within a native plant population area in Llombai (Valencia, Spain). The microsymbionts are extra-slow (ultrabradytrophic) growing bacteria with phenotypic and symbiotic characteristics singularly different from *Bradyrhizobium* strains nodulating other *Lupinus* spp. thriving in the Iberian Peninsula and adapted to growth in acidic soils. Cross-inoculation experiments revealed that these *L. mariae-josephi* endosymbiotic bacteria isolates are unable to nodulate or efficiently fix nitrogen with other *Lupinus* spp. Their phylogenetic status was examined by a multilocus sequence analysis of four housekeeping genes (16S rDNA, *glnII, recA, atpD*) and the symbiotic *nodC* gene. The 16S rDNA phylogenetic analysis showed that *L. mariae-josephi* isolates are related to strains nodulating *Retama* spp. in northeastern Algeria (Boulila et al., 2009), *Phaseolus lunatus* from Peru (Ormeño-Orrillo et al., 2006), as well as to *B. elkanii, B. jicamae* and *B. pachyrhizi* species, forming a new clade (Clade I) within the *Bradyrhizobium* genus. All the single and concatenated *glnII+recA* and *glnII+recA+atpD* analyses consistently support the existence of Clade I, and also revealed that, within this clade, the *L. mariae-josephi* endosymbiotic bacteria belong to a single evolutionary lineage that also includes strains nodulating *Retama* spp. from northeastern Algeria. Within this new *Bradyrhizobium* lineage, the phylogenetic analyses performed showed essentially convergent results indicating that the tested *L. mariae-josephi* isolates nested in three sub-groups that might correspond to novel sister *Bradyrhizobium* species. *Bradyrhizobium* Clade I is highly differentiated from the *Bradyrhizobium* clade (Clade II) that includes currently named *Bradyrhizobium* species and well-delineated unnamed genospecies. Singularly, all the endosymbiotic bacteria from *Lupinus* species adapted to acid soils in the Iberian Peninsula and

tested in this study are included in Clade II. They are related either to strains of the *B. canariense or B. japonicum* lineages. The phylogenetic analysis based on the symbiotic *nodC* gene showed that *L. mariae-josephi* endosymbiotic bacteria define a novel branch in the *nodC Bradyrhizobium* tree. This branch groups together with a branch that gathers isolates from recently studied legume symbiosis such as isolates from *Retama* spp., which suggests the existence of a common unique ancestor for the symbiotic genes of these two groups of bradyrhizobia. In contrast, the symbiotic genes of isolates from other *Lupinus* spp. from the Iberian Peninsula are clearly related to the *B. canariense* lineage. The allopatric (geographic) speciation of the *L. mariae-josephi* bradyrhizobia may result from the colonization of a singular habitat, such as the basic and high calcium carbonate soils of the Valencia area, by its unique legume host.

References

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